

AMENDMENT

In the Specification:

Please add the attached Sequence Listing, and amend paragraphs 28, 29, 150, 151, 152, 153, 155, and 163 as indicated in the listing of specification amendments attached hereto pursuant to 37 C.F.R. §1.121.

Specification Amendments In the Application Pursuant to 37 C. F. R. § 1.121

[0028] FIG. 8 sets forth the nucleotide sequence of ATF5 (SEQ ID NO: 1).

[0029] FIG. 9 sets forth the amino acid sequence of ATF5 (SEQ ID NO: 2).

**EXAMPLE 3 – CLONING OF FULL-LENGTH rATF5 AND
PLASMID CONSTRUCTS**

[00150] SAGE tag, CATGAGAACCTAGTC (SEQ ID NO: 3), was found in rat EST, UI-R-G0-ur-g-10-0-UI (GenBankTM/EBI accession number AI576016), which, in turn, showed high homology with the 3' end of murine ATF5. To clone the open-reading frame of rat ATF5, PCR antisense primer 5'-CTTGGTTCTCAGTTGCAC-3' (derived from the sequence of the above EST) (SEQ ID NO: 4) was used for 5' RACE PCR, using the Clontech Marathon kit according to the manufacturer's protocol. The first-strand cDNA PCR template was prepared from 5 µg of PC12 cell total RNA, by reverse transcription with Superscript II. The products of the 5' RACE PCR included the second of 2 potential Kozak start sites.

[00151] Cloning of the rATF5 open-reading frame that included the first potential start site was achieved with sense PCR primer, 5'-TGCACCTGTGCCCTAGCCATGTC-3' (SEQ ID NO: 5). This sequence was obtained from an EST sequence (GenBankTM/EBI accession number AW917099) that overlapped with the 5' end of the 5' RACE PCR product described above. Both potential rATF5 forms were FLAG-tagged, by PCR, with sense primers, 5'-CTCGAGA

ACCATGGACTACAAGGACGATGATGACAAAGGATCACTCCTGGCGACCCT-3'
(SEQ ID NO: 6), and 5'-

CTCGAGAACATGGACTACAAGGACGATGATGACAAAGGAGCATCC
CTACTC AAGAA-3' (SEQ ID NO: 7). 5'-

GAATTCTCGAGCTTGGTTCTCAGTTGCAC-3' (SEQ ID NO: 8) was the antisense primer for both ATF5s. NTAzip-ATF5 was constructed by overlapping PCR, using FLAG-tagged ATF5 (potential start site 2 form) as the template.

[00152] PCR product 1 was produced with 5'-CTCGAGAACATGGACTACAA
GGACGATGATGACAAAGGAGCATCCCTACTCAAGAA-3' (SEQ ID NO: 9) and 5'-
TTCTTCTGCT

TCTTTTCTAGTAGTCTCGTTTCTCTGCTAGTTCTGCTCTT
GTTCGAGGGTGCTGGCAGGACTAGGATA-3' (SEQ ID NO: 10) as primers, and PCR
product 2 was made with 5'-GC
AAGAGAAAACGAAGAACTACTAGAAAAAGAACGAGAAGAACTAGAACAAAG
AAATGCAGAGCTAGAGGGCGAGTGCCAAGGG-3' (SEQ ID NO: 11) and 5'-
GAATTCTCGAGCTTG GTTTCTCAGTTGCAC-3' (SEQ ID NO: 12) as primers. Products
1 and 2 were mixed, and the product (FL-NTAzip-ATF5) was PCR amplified with 5'-
CTCGAGAACATGGACTACAAGGACGAT
GATGACAAAGGAGCATCCCTACTCAAGAA-3' (SEQ ID NO: 13) and 5'-
GAATTCTCGAGCTTGGTTT CTCAGTTGCAC-3' (SEQ ID NO: 14). To generate
NTAzip-ATF5, the activation domain was removed from FL-NTAzip-ATF5 by PCR, using
primers 5'-GAATTCAACCATGGACTACAAGGACGA
TGATGACAAAATGGCATCTATGACTGGAGGACAACAAATGGGAAGAGACCCA
GACCTCGAACAAAGAGCAGAA-3' (sense) (SEQ ID NO: 15) and 5'-
GAATTCTCGAGCTTGG
TTTCTCA GTTGCAC-3' (antisense) (SEQ ID NO: 16).

[00153] NTAzip-ATF5 was N-terminal FLAG-tagged with a predicted open-reading frame of MDYKDDDDKMASMTGGQQMGRDPDLE**QRAEELRENEELLEKEAEEELE**
QENAELEGECEQGLEARNRELRERAESVEREIQYVKDLLIEVYKARSQRTRSA (SEQ ID NO: 17), where the DNA binding motif was replaced with an amphipathic acidic α -helical sequence, as marked in bold (Moll *et al.*, Attractive interhelical electrostatic interactions in the proline- and acidic-rich region (PAR) leucine zipper subfamily preclude heterodimerization with other basic leucine zipper subfamilies. *J. Biol. Chem.*, 275:34826-832, 2000). All PCR products were subcloned into the Topo II pCR 2.1 vector, and were sequenced to verify identity. After confirmation, all full-length constructs were subcloned into the EcoR1 sites of the pCMS-eGFP vector.

[00155] The CRE-luciferase reporter plasmid was constructed by annealing synthetic oligo 5'-TCGAGTCATGGAAAAATGACGTCAATTATCATGGTAAAAAT
GACGTCAATTATCATGGTAAAAATGACGTCAATTATCATGGTATTA-3' (SEQ ID NO: 18) to 5'-AGC TTAATTACCATGACGTCAATTACCATGATAATTACCATGACGTCA
TTTACCATGATAATTACCATGACGTCAATTACCATGAC-3' (SEQ ID NO: 19), to

form a double-stranded DNA (Peters *et al.*, ATF-7, a novel bZIP protein, interacts with the PRL-1 protein-tyrosine phosphatase. *J. Biol. Chem.*, 276:13718-26, 2001). The annealed DNA was ligated into the XhoI and HindIII sites of the GL3 plasmid. VP16-CREB (Columbia University) was subcloned into the EcoRI and XbaI sites of the pCMS-eGFP vector.

EXAMPLE 8 – TRANSIENT TRANSFECTIONS

[00163] For PC12 cells, transfection was carried out using 0.5 µg of plasmid / well and 6 µl / well of LipofectAMINE 2000, for 9 h. Thereafter, the cells were re-fed with fresh culture medium, and then handled as described. For telencephalic cells, transfection was performed with 2.0 µg of plasmid / well and 2 µl / well of LipofectAMINE 2000 for 7 h followed by an exchange of medium. For transfection of ATF5 siRNA (AAN19; AAG UCA GCU GCU CUC AGG UAC, (SEQ ID NO: 20))), 6.67 µg / well of pCMS-EGFP vector were mixed with 80 pmol / well of siRNA in 100 µl of DMEM medium. An equal amount of DMEM medium, premixed with 1 µl of LipofectAMINE 2000 / well, was added to, and mixed with, the vector and siRNA. After 30 min, the final mixture was added to 1/6 the volume containing the cells, and the cells were re-fed with fresh culture medium after 7 h of transfection. For the control, telencephalic cells were transfected with pCMS-EGFP vector alone.